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Diagnostic value of complement components in pleural fluid: Report of 135 cases

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Summary
We prospectively assessed the diagnostic value of pleural fluid complement levels (total, C3, C4) in 135 patients with pleural effusion of five main etiologies, using novel markers. Complement levels correlated with pleural levels of protein, amylase, and transuded fluids. On univariate analysis, CHF-related pleural effusions were associated with significantly lower C4 levels than postsurgery or parapneumonic effusions. On multivariate analysis, pleural fluid C4 level was a significant predictor of CHF. Although the specificity, positive predictive value, and accuracy of the parameters were low in all diagnostic groups, their negative predictive value as well as the AUC ROC was high for CHF and post-LTX. We conclude that pleural fluid C4 levels can differentiate CHF-related pleural effusion from other etiologies and that normal level of C3 or C4 rule out CHF or LTX as causes of pleural effusion. Complement should be included in the assessment of pleural effusion when traditional diagnostic methods fail.

Introduction

Pleural effusion is a common complication of a variety of local and systemic diseases. The most common causes are malignancy, infection, tuberculosis, pulmonary embolism, congestive heart failure (CHF), and systemic autoimmune disease.[1] In about one-third of cases, the cause remains unknown, despite thorough examination.[2]

Complement activation may be measured by monitoring the total complement and the C3 or C4 components of the cascade.[3] Several studies have reported the activation of complement in the pleural fluid of patients with rheumatoid arthritis[4–7] and tuberculosis.[8,9] However, the association between complement activation and the cause of pleural effusion has not been thoroughly examined.

By measuring concentrations both of various components of complements and of their activation products, it is possible to decide which pathway is prevailing. High levels

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of C4activation product (C4d) reflect classic pathway activation, and high levels of factor B activation product (Bb) reflect alternative pathway activation. When either the classic or the alternative pathway is activated, the concentrations of C3activation product (C3d) and C5b–9 are increased. Detection of complement complexes or fragments in pleural fluids directly indicates complement activation.

The aim of this study was to determine the value of complement component measures in determining the underlying cause of pleural effusion.

Patients and methods

Patients

The study population consisted of 135 patients, 79 men (59%) and 56 women (41%), with pleural effusion who attended the Pulmonary Institute of Rabin Medical Center from January 2005 to December 2006. The diagnoses were divided into five categories on the basis of the underlying disease/procedure.

– The diagnosis of malignant effusion was made when malignant cells were found on cytologic examination or in a biopsy specimen. Patients with negative cytologic findings were further examined with closed pleural biopsy, and those with negative findings on closed biopsy underwent video-assisted thoracoscopic surgery.

– The pleural effusion was considered parapneumonic if it was associated with acute febrile illness with purulent sputum, pulmonary infiltrate, and responsiveness to antibiotic treatment, or if the microorganism was identified in the pleural fluid in the absence of any other cause of the pleural effusion. Tuberculous pleurisy was diagnosed by positive cultures for *Mycobacterium tuberculosis* or when the pleural biopsy specimen showed typical epithelioid cell granuloma.

– The pleural effusion was attributed to CHF in patients in whom CHF was diagnosed by findings of an enlarged heart, pulmonary venous congestion on the radiograph, and peripheral edema, with response to CHF treatment and absence of malignancy or pulmonary infiltrates associated with an inflammatory process.

– The diagnosis of postcardiotomy or postsurgery (Dressler's syndrome) pleural effusion was made when the pleural fluid developed after injury to the heart, the patient responded to treatment with anti-inflammatory agents or corticosteroids, and CHF, pulmonary embolism, and pneumonia were ruled out.

– The diagnosis of post-LTX pleural effusion was made in patients who had undergone lung transplantation and had no evidence of infection, rejection or other known cause of pleural effusion.

Laboratory studies

Pleural fluid was collected from each patient prior to any therapy for biochemical, cytological and microbiological analysis. Supernatant was obtained by centrifugation at 300 rpm for 15 min and stored at $-20\,^{\circ}C$ until assayed. The clinicians who performed the laboratory studies were blinded to the diagnosis of the pleural effusions.

The tumor markers carcinoembryonic antigen (CEA), cytokeratin fragment (CYFRA) 21–1, cancer antigen (CA) 19–9, CA15–3, and CA 125 were measured by solid-phase, two-site chemiluminescent enzyme immunometric assays. Cutoff levels were as follows: CEA, 0–5 ng/ml; CYFRA 21–1, 0–3.3 ng/ml; CA15–33, 0–30 U/ml; CA 19–9, 0–37 U/ml; CA 125, 0–35 U/ml.

Total complement and C3 and C4 levels in pleural fluid were assayed in duplicate using commercially available sandwich ELISA kits supplied by Progen Biotechnik (Heidelberg, Germany).

Statistical analysis

Results are shown as mean ± standard deviation. Pearson correlation coefficient (r) and the significance for it (p) were calculated between the variables. To analyze differences in the distribution of categorical data, chi-square test or Fisher exact test was used, as appropriate.

Sensitivity, specificity, positive and negative predictive values (PPV and NPV), and accuracy of the complement component levels were calculated between patients with and without malignancy. Accuracy was defined as (true–positive + true–negative)/(true–positive + false–positive + true–negative + false–negative).[10] The predictive capability of each complement variable was demonstrated by a receiver operator characteristic (ROC) curve. The area under the curve (AUC) was calculated as a measure of discriminative ability. The higher the AUC values the better discriminatory ability, as follows: AUC of ≥ 0.90 –excellent discrimination; $0.80 \leq \text{AUC} < 0.90$ – good discrimination; $0.70 \leq \text{AUC} < 0.80$ – fair discrimination; and $\text{AUC} < 0.70$ – poor discrimination. Differences in mean continuous variables between the two groups of patients (with and without malignancy) were analyzed by t-test.

To predict malignancy a stepwise logistic regression was fitted to the data. Odds ratio and 95% confidence interval were calculated from the model.

A p value of 0.05 or less was considered statistically significant.

Results

Table 1 summarizes the clinical and laboratory characteristics of the whole study population ($n = 135$) and by diagnostic group.

No significant differences were noted among the groups in patient age and sex distribution. The malignancy and post-LTX groups had predominantly exudative pleural fluid. The levels of all the pleural markers were significantly higher in the malignancy group than in the other diagnostic groups.

The mean concentrations of pleural complement and its components (C3, C4) were in the low range of normal in all the diagnostic groups (Table 2), although they were lowest in the patients with CHF and after LTX. In addition, significant correlations were noted for the presence of CHF with C3 level ($r = -0.19$, $p = 0.04$) and C4 level ($r = -0.23$, $p = 0.01$).

On univariate analysis, mean pleural fluid C4 level was significantly lower in patients with CHF than in patients

Table 1 Clinical characteristics of the study population ($n = 135$)

	All	Malignancy	Para-pneumonia	CHF	Postsurgery	LTX
	$n = 135$	$n = 56$	$n = 23$	$n = 20$	$n = 15$	$n = 15$
Sex (F/M)	56/79	26/30	11/12	5/10	6/9	7/8
Age, years ± SD	67 ± 14	67 ± 14	68 ± 15	66 ± 14	67 ± 16	58 ± 125
Median	67.0	71.5	74.0	67.0	64.0	61.5
Protein, gr%	4.2 ± 1.1	4.6 ± 0.7	4.7 ± 0.9	3.1 ± 1.2	4.7 ± 0.9	2.8 ± 0.6
Median	4.4	4.65	4.9	2.8	4.8	3.0
LDH, mg%	533 ± 622	525 ± 412	984 ± 1210	189 ± 120	497 ± 548	448 ± 237
Median	355.5	374	489.0	158	301.5	359
Glucose, mg%	121 ± 47	109 ± 34	110 ± 42	119 ± 28	129 ± 47	187 ± 73
Median	113	105	106.0	112	121	153
Amylase, mg%	42 ± 22	48 ± 23	36 ± 14	31 ± 13	43 ± 18	33 ± 35
Median	37	45.5	35.5	33	41	23
Trans/exudative (*n*)	26/109	1/55	2/22	13/7	2/13	8/7
CEA, U/L	88 ± 422	203 ± 640	1.2 ± 0.8	0.9 ± 0.6	0.8 ± 0.5	2.4 ± 2
Median	1.3	3	0.9	0.8	0.6	1.7
CYFRA 21-1, ng%	91 ± 154	143 ± 195	107 ± 159	21 ± 24	22 ± 27	47 ± 78
	18.9	29.4	22.8	14.2	16.5	23
CA 15-3, ng%	85 ± 383	181 ± 578	16 ± 8	9 ± 11	12 ± 9	10 ± 5
Median	11.7	20.6	14.55	5.6	8.7	9.9
CA 125, ng%	1231 ± 4581	2099 ± 6997	748 ± 636	652 ± 639	523 ± 473	308 ± 421
Median	491.6	849	418.3	535	260	
CA 19-9, ng%	483 ± 4384	1130 ± 6807	1.4 ± 19	3.2 ± 7	4.9 ± 5.9	5.8 ± 14
Median	0.9	1.9	0.6	0.1	3.7	2.7

All data are mean ± SD and median. CHF = congestive heart failure, LTX = lung transplantation, LDH = lactate dehydrogenase, CA = cancer antigen, CEA = carcinoembryonic antigen, CYFRA = cytokeratin fragment.

with postsurgery or parapneumonic pleural effusions ($p = 0.04$; $p = 0.01$, respectively). On multivariate regression model, only C4 was a significant predictor of CHF.

Table 3 shows the Pearson correlation coefficient (r) and the significance for it between complement and clinical characteristics. C3, C4 and total complement levels all correlated best with total protein levels, of all the characteristics examined (C3, $r = 0.6$, $p = 0.001$; C4, $r = 0.56$, $p = 0.001$; total C, $r = 0.42$, $p = 0.001$).

Significant correlations were noted between complement components and amylase level, tranudate/exudate data and protein level in pleural fluid (Table 3).

Table 4 showed the sensitivity, specificity, NPV, PPV, and accuracy of C3, C4 and total complement measurements in the various diagnostic groups as well as the AUC ROC curve for no disease. Although the specificity, PPV, and accuracy of the three parameters were low in all the diagnostic groups, their negative predictive value was high for CHF and post-LTX pleural effusion (C3, 92% for CHF and 96% for LTX; C4, 94% for both; total complement, 90% for both). A relatively high NPV was also found for postcardiotomy effusion (C3, 84%; C4, 83%; total complement, 88%). A relatively high AUC ROC curves for no diseases were also found for CHF and postcardiotomy effusion (Table 4).

The stepwise regression model showed that none of the parameters predict malignancy.

Discussion

In clinical practice, clinicians often find it difficult to identify the cause of pleural effusions. The present study demonstrates that regardless of the properties of the fluid

Table 2 Complement levels by diagnostic groups

	Total	Malignancy	Parapneumonia	CHF	Postsurgery	LTX
	$n = 135$	$n = 56$	$n = 23$	$n = 20$	$n = 15$	$n = 15$
C3	53.3 ± 26	54.5 ± 23	58 ± 24	41 ± 34	58 ± 9	44.8 ± 29
	48.0	50.0	60	31.0	50.0	34.0
C4	12.8 ± 7.2	13.4 ± 7.3	14.8 ± 7.4	9 ± 7.8	14 ± 5.9	10.7 ± 5
	12.0	12.0	13.0	6.0	13.0	10.0
Total C	38.8 ± 16	39.2 ± 14	42 ± 21	31 ± 14	38 ± 17	37.2 ± 14
	38.0	38.0	40.5	29.5	32.0	31.0

All data are mean ± SD and median. CHF = congestive heart failure, LTX = lung transplantation.

Table 3 Pearson correlation coefficient (r) and the significance for it (p) between complement components and clinical variables ($n = 135$)

	C3	C4	Total C
Protein	$r = 0.6$ $p = 0.001$	$r = 0.56$ $p = 0.001$	$r = 0.42$ $p = 0.0001$
Amylase	NS	$r = 0.21$ $p = 0.04$	$r = 0.25$ $p = 0.02$
Transudate/ exudates	$r = -0.37$ $p = 0.0001$	$r = -0.39$ $p = 0.001$	$r = -0.25$ $p = 0.01$

CHF = congestive heart failure.

(transudative or exudative), measurement of the complement could add important diagnostic data.

Earlier studies found that the activation of complement components in the pleural fluid is associated with autoimmune diseases and infections[4,6,11,12] Specifically, most of the patients with pleural effusions secondary to SLE or RA had reduced levels of pleural fluid complement — total (CH50) and C3 or C4 components.[13,14] Although the findings did not definitively differentiate patients with SLE or RA from patients with exudative pleural effusions of other causes, it was noteworthy that a CH50 level below 10 U/ml, or a C4 level below 10 times 10^{-5} U/g protein, was detected in most of the patients with autoimmune disease and rarely in the patients with other diseases. Nevertheless, complement levels are no longer recommended to identify RA- or SLE-related pleural effusions because serum antibody to nuclear antigen (ANA) levels and rheumatoid factor (RF) titers appear to be more specific and more sensitive.[15]

Several recent studies evaluated the diagnostic utility of measuring complement activation products in pleural fluid. Two found that levels of SC5b—9, a product of C3 activation, are elevated in tuberculous pleural effusions as opposed to malignant pleural effusions or transudates, and that SC5b—9 levels are highest in patients with rheumatoid diseases.[8,16] Specifically, Hara et al.[8] reported that all patients with tuberculous effusions had SC5b—9 levels above 2 mg/L, whereas those with malignant effusions had levels below 2 mg/L. Using this value as the cut-off, they found that the specificity of the estimated SC5b—9 level for tuberculosis-related effusion was 74%, and the sensitivity, 100%. In addition, there was a correlation between adenosine deaminase (ADA) and SC5b—9 values in the pleural effusions.

In the second study, Salomaa et al.[16] found that the SC5b—9 level in the pleural fluid was higher than 2 AU/ml in all their patients with rheumatic disease and lower than 2 AU/ml in all their patients with malignancy. Furthermore, the concentrations of pleural fluid C3 and C4 were significantly lower, and the ratio of C4d/C4 significantly higher, in the patients with rheumatic pleurisy than in those with tuberculous or malignant pleurisy. However, the overlap among the various groups, the relatively small number of patients with rheumatic effusions, and the contribution of ADA in differentiating tuberculous from other effusions limited the diagnostic usefulness of this test.

Therefore, in the present study, we sought to clarify the diagnostic value of these products using novel markers. To the best of our knowledge, this is the only study to assess complement components in pleural effusions of all known etiologies, including transudative fluid (as in CHF). In addition, LTX is nearly always associated with pleural effusion, usually exudative and bloody, and our study is the first to assess the pleural complement component profile in patients after LTX, adding important information on its clinical implications.[17]

Our analyses yielded several noteworthy observations. First, transudative fluids are apparently characterized by low levels of complement and its components. This finding

Table 4 Sensitivity, specificity, negative and positive predictive values and accuracy (in %) of C3, C4 and total complement by diagnostic group and AUC ROC for no disease

	Diagnostic group	Sensitivity	Specificity	NPV	PPV	Accuracy	AUC ROC for no disease
C3	Malignancy	86	22	68	44	49	0.435
	Parapneumonia	68	16	72	14	25	0.359
	CHF	90	20	92	17	31	0.731
	Postsurgery	73	18	84	10	24	0.367
	Post-LTX	93	22	96	13	30	0.738
C4	Malignancy	75	28	61	43	48	0.442
	Parapneumonia	61	24	75	14	31	0.337
	CHF	90	30	94	18	39	0.741
	Postsurgery	60	25	83	9	29	0.408
	Post-LTX	86	29	94	13	35	0.678
Total C	Malignancy	61	37	57	41	47	0.459
	Parapneumonia	48	35	76	13	37	0.419
	CHF	75	40	90	18	46	0.673
	Postsurgery	60	38	88	11	40	0.536
	Post-LTX	67	39	90	12	42	0.540

CHF = congestive heart failure, LTX = lung transplantation, PPV = positive predictive value, NPV = negative predictive value.

was supported by the correlation of pleural fluid complement levels and total protein and amylase levels. Second, CHF-related pleural effusions can be differentiated from parapneumonic and postsurgery pleural effusions by the low level of complement. Third, a finding of normal levels of complement almost completely rule out CHF as an etiologic factor. Fourth, in patients after LTX, findings of normal or high pleural fluid complement levels may indicate that the lung effusion is not attributable to the surgery, but to another secondary cause (such as parapneumonic effusions).

On the basis of these findings, we suggest that pleural complement components should be included in the assessment of pleural effusion when traditional diagnostic methods fail.

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